## REMARKS

After entry of the amendments made herein, claims 1 – 13 are pending in the application. Claim 1 has been amended. Claims 2, 3, and 14 - 29 have been cancelled. New claims 30 – 32 have been added. No new matter has been added by virtue of the amendments, support being found throughout the specification and from the claims as filed.

Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

### Objections

The Examiner has indicated that the disclosure has been objected to because it contains an embedded hyperlink and/ or other form of browser-executable code. Applicants have amended the specification to delete the embedded hyperlink and respectfully request that the objection be withdrawn.

### Information Disclosure Statement

The Examination has indicated that the IDS filed 12/07/06 has been considered.

### Claim Rejections 35 USC 112

The Examiner has indicated that claims 1 – 13 have been rejected under 35 USC 112, first paragraph, because the specification, while being enabling for 1) a method of reducing myotonia in the muscle of an individual suffering from myotonia, comprising intramuscular injection of a recombinant adeno-associated viral (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding muscleblind (MBNL1) protein, wherein expression of the MBNL1 protein results in reducing myotonia in the muscle of the individual, and 2) a pharmaceutical composition comprising a recombinant adeno-associated viral (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding mucleblind (MBNL1) protein, but the specification does not reasonably provide enablement for 1) the breadth of treatment of any disease associated with aberrant microsattelite expansion; 2) the breadth of any type of treatment of any disease associated with aberrant microsattelite expansion; 3) utilizing

rAAV, not an rAAV vector; 4) any mode of administration of rAAV; 5) utilizing any combination of transgenes encoding for MBNL1, MBNL2, MBNL3 and 6) the reversing of mis-splicing of variously claimed proteins in vivo. Applicants respectfully disagree.

The present claims recite a method of treating myotonic dystrophia in a subject, comprising administering to a mammal in need thereof, a therapeutically effective amount of recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding a protein selected from the group consisting of: MBNL1, MBNL2 and MBNL3 protein, wherein expression of the protein results in reducing myotonic dystrophia in the subject.

The Examiner argues that the specification does not reasonably provide enablement for "the breadth of treatment of any disease associated with aberrant microsattelite expansion." (Office Action, p.3). The Examiner argues that "the breath of any type of treatment of any disease associated with aberrant microsattelite expansion" is not enabled.

Applicants have amended the claims to recite treating myotonic dystrophia (DM) in a subject. The specification provides ample teaching to enable the method as claimed. Applicants characterize a Mbnl1 knockout mouse to show that sequestration of MBNL1 contributes to DM pathogenesis. (see Example 1). As pointed out by the Examiner, Applicants teach that "Example 2 teaches that MBNL1 knockout mice displayed overt myotonia, delayed muscle reaction, wherein a similar 'warm up' phenomenon is characteristic of myotonia in human DM." (Office Action, p.5). The Examiner also points out on page 5 of the Office Action that Applicants teach that "besides muscle abnormalities the Mbnl1 knockout mice developed cataracts, which is a prominent DM-associated feature in humans." The Examiner notes Applicant's Example 3 that teaches "heart tissues from Mbnl1 knockout mice shows the abnormal retention of the Tnnt2 fetal exon 5, which is observed for DM1." (Office Action, p.5). The Examiner points out Example 4, where the left tibialis anterior muscle of mice were injected with a recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding MBNL1 protein (rAAVMyc-hMBNL1) and an then analyzed, the results showed that "overexpression of MBNL1

showed virtual elimination of myotonia in the injected tibia muscle." (Office Action, p.6). Taken together, Applicants have shown that the specification provides enablement for the treatment of a disease associated with aberrant microsattelite expansion, where the disease is myotonic dystrophia.

The Examiner argues that the specification does not reasonably provide enablement for "utilizing an rAAV (i.e. a virus) not an rAAV vector." (Office Action, p.3).

Applicants have amended the claims such that the claimed method comprises administering to a mammal in need thereof, a therapeutically effective amount of recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding MBNL1 protein. Recombinant AAV (rAAV) vectors are described in the specification, for example at page 12 beginning at line 12.

Applicants teach at line 13 that "recombinant AAV (rAAV) vectors have been used for expressing gene products in animals, see, for example, U.S. Pat. No. 5,193,941 and WO 94/13788. Other patents and publications describe AAV vectors and uses, the uses generally being related to expression of gene products either in vitro (usually tissue cultures) or in vivo (usually in the lungs or oral mucosa, the normal sites of AAV infection, but expression in other tissues, such as the central nervous system and in cardiac tissue has been observed)."

The Examiner argues that the claimed invention is gene therapy...(and) numerous factors complicate somatic gene therapy with respect to predictability achieving levels and duration of gene expression." (Office Action, p.8). The Examiner cites the Patil et al. reference, the Juengst et al. reference, the Kay et al. reference and the Gregorevic et al. reference to support his argument that "the art of gene therapy, and in particular muscle gene therapy, is not found to be predictable." (Office Action, p.10).

Applicants teach that rAAV vectors have been used for the successful expression of a number of genes in vitro:

> Transduction of rAAV vectors harboring the bacterial beta-galactosidase gene by single injection into the

> quadriceps of mice demonstrated that expression was maintained long-term and the expression did not decrease substantially during that time (Xiao et al., J. Virol., 70:8098-8108 (1996)). Other targets successfully transduced with rAAV vectors include: T-lymphocytes and B-lymphocytes, human erythroleukemia cells, different regions of the rat brain, the striatum of the rat brain in a Parkinson's Disease model with the tyrosine hydroxylase gene, heart of the pig and rat with the LacZ gene, the peripheral auditory system of the guinea pig and bronchial epithelia of the rabbit and monkey. (page 12, line 26 – 32).

The Examiner argues that "Patil teach that the particular delivery (including vector and route of administration) is paramount to a success treatment using gene therapy." (Office Actin, p.9). Applicants point out that the present invention does teach a particular vector, rAAV, for gene expression and , and does teach intramuscular injection of the rAAV-construct into a transgenic model for DM. Applicants direct the Examiner to Example 4 beginning on page 30, where the left tibialis anterior muscle of mice was injected with PBS containing a rAAV1 MBNL1 construct.

The Examiner argues that the Juengst et al. reference (2003) and the Kay et al. reference (2001) teaches "the unpredictability in the gene therapy art, and the requirement for specific targeting and specific design." (Office Action, p.9). The Examiner also argues that the Gregorovic reference (2003) teaches "that the challenges faced with developing an intervention capable of curing even a single form of muscular dystrophy remains formidable (and) teaches in vivo muscle gene therapy must provide a sufficient efficiency for therapeutic gain and be able to specifically target muscles."' (Office Action, p.10).

The references cited by the Examiner are 6 – 8 years old. The field of gene therapy has changed considerably in this time. In particular, Applicants point out that in 2000, a Phase I clinical trial was conducted utilizing gene therapy for limb girdle muscular dystrophy (Stedman et al., Hum Gene Ther. 2000 Mar 20;11(5):777-90, provided herein). Further, a recent gene therapy trial in muscular dystrophy has shown the safe transfer of a gene to produce a protein necessary for healthy muscle fiber

growth into three teenagers with limb-girdle muscular dystrophy. (Clemens et al. Annals of Neurology, 20 July 2009; provided herein).

Clearly, Applicants have shown that the specification provides enablement for using an rAAV vector in the methods as claimed.

The Examiner argues that the specification does not reasonably provide enablement for "any mode of administration of the rAAV." (Office Action, p.5).

It is known in the art that rAAV can be administered by many methods. For example, the specification teaches at page 12, line 28, that targets successfully transduced with rAAV vectors include: T-lymphocytes and B-lymphocytes, human erythroleukemia cells, different regions of the rat brain, the striatum of the rat brain in a Parkinson's Disease model with the tyrosine hydroxylase gene, heart of the pig and rat with the LacZ gene, the peripheral auditory system of the guinea pig and bronchial epithelia of the rabbit and monkey. The specification teaches that the rAAV vectors may be administered intravenously, parenterally or intraperitoneally (see, e.g. page 16, line 23).

In Example 4 beginning on page 30, the left tibialis anterior muscle of mice was injected with PBS containing a rAAV1 MBNL1 construct. In this set of experiments, the mice were tested for myotonia, and the results show that injection of the mice with rAAV1Myc-hMBNL1 into the tibialis anterior results in reduced myotonia in as little as four weeks' time. (page 31, line 13 – 14).

Accordingly, Applicants have shown that the specification provides enablement for administering the rAAV in the methods as claimed.

The Examiner argues that the specification does not reasonably provide enablement for "utilizing any combination of transgenes encoding for MBNL1, MBNL2, MBNL3." (Office Action, p.5).

The present claims are directed to a method of treating myotonic dystrophia in a subject, comprising administering to a mammal in need thereof, a therapeutically effective amount of recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding a protein selected from the group consisting of: MBNL1, MBNL2 and MBNL3 protein.

The Examiner indicates on page 12 of the Office Action that the specification "provides guidance to show that a transgene encoding for MBNL1 would provide the effect of reducing myotonia in a mouse having myotonia." The Examples clearly support a role for MBNL in DM. Applicants characterize a Mbnl1 knockout mouse to show that sequestration of MBNL1 contributes to DM pathogenesis. (see Example 1). Example 2 teaches that MBNL1 knockout mice displayed overt myotonia, delayed muscle reaction, similar to myotonia in human DM. Applicants teach in Example 2 that Mbnl1 knockout mice developed cataracts, which is a prominent DM-associated feature in humans.

Applicants point out that the specification also provides support for treating myotonic dystrophia by administering a rAAV vector comprising a promoter operably linked to a nucleic acid encoding MBNL2 or MBNL3 protein.

As taught by Applicants in the present specification, "myotonic dystrophy type 1 (DM1) is caused by a trinucleotide $(CTG)_n$ expansion (n=50 to >3000) in the 3'-untranslated region (3'UTR) of the Dystrophia myotonica-protein kinase (DMPK) gene. Myotonic dystrophy type 2 (DM2) is caused by a tetranucleotide $(CCTG)_n$ expansion (n=75 to .about.11,000) in the first intron of zinc finger protein 9 (ZNF9) gene (Ranum, L P W, Day, J W (2002) Curr. Opin. in Genet. and Dev. 12:266-271)." (page 3, line 10). However, as pointed out by the Applicants at page 4, line 8, "the mechanism by which expanded repeats alter the regulation of pre-mRNA alternative splicing is unclear. Two families of RNA-binding proteins have been implicated in DM1 pathogenesis: CUG-BP1 and ETR-3-like factors (CELF) and muscleblind-like (MBNL) proteins (Ladd A N, Charlet-B N, Cooper T A (2001), Mol Cell Biol 21: 1285-1296)."

In the Examples, Applicants show that MBNL 1, 2 and 3 all regulate splicing of mRNA targets known to be abnormally regulated in DM.

For example, FIG. 6 shows the results of RT-PCR analysis of exon inclusion. As described at page 32, beginning at line 24, GFP-**MBNL1, 2 and 3** strongly repressed inclusion of both human and chicken cTNT exon 5 in primary chicken skeletal muscle cultures, while expression of GFP to levels comparable to, or greater than, GFP-MBNL fusion proteins had no effect on splicing (see FIGS. 6A and 6B).

Further, as described at page 32 beginning at line 34, to test whether the MBNL family can also regulate human IR, the three MBNL family members were co-expressed

with a human IR minigene. In contrast to the inhibitory effect of MBNL on cTNT splicing, **coexpression of MBNL family members** with an IR minigene strongly induced exon inclusion, whereas GFP alone had no effect (see FIG. 6C). Moreover, as described at page 33, beginning at line 4, to determine whether the MBNL family has a general effect on alternative splicing, all three MBNL proteins were co-expressed with a clathrin light-chain minigene containing the neuron-specific exon EN. Applicants show that over-expression of GFP-MBNL1, 2 and 3 with the clathrin light-chain minigene had no effect on alternative splicing of exon EN (see FIG. 6D). Applicants show that MBNL1, 2 and 3 regulate splicing of cTNT and IR alternative exons.

Alternative splicing by all three MBNL proteins is known in the art, as shown by the attached references. Ho et al. (The EMBO Journal (2004) 23, 3103 – 3112, provided herein) teach that a key feature of the RNA-mediated pathogenesis model for DM is the disrupted splicing of specific pre-mRNA targets, and present data that MBNL proteins regulate alternative splicing of two pre-mRNAs that are misregulated in DM. In particular, Ho et al. show that **all three MBNL family members** are novel splicing regulators that act antagonistically to CELF proteins on the three pre-mRNAs tested: human and chicken cTNT, and human IR, that is all three MBNL proteins can alter the splicing patterns of pre-mRNAs known to be abnormally regulated in DM1 striated muscle.

Accordingly, the specification provides enablement for the claims as written.

The Examiner argues that claims 4 – 8 are not enabled. The Examiner argues that "the claims are directed to 'reversing' the mis-splicing of the Clcn1 skeletal muscle chloride channel, reversing the mis-splicing of the Amyloid beta (A4) precursor protein (APP), reversing the mis-splicing of the NMDA receptor NR1 (GRIN1), reversing the mis-splicing of the Microtubule-associated protein tau (MAPT), reversing the mis-splicing of the TNNT2 (cTNT) gene... however the specification provides not specific guidance to show that administration of an rAAV vector containing a transgene encoding either MBNL1, MBNL2, or MBNL3 or combinations thereof, would result in reversing of the missplicing of these proteins" (Office Action, p.13). Applicants disagree.

Applicants direct the Examiner to Example 4 beginning at page 30 that shows AAV-MBNL1 Injection and Clcn1 splicing. In these experiments, the left tibialis anterior muscle of mice were injected with a recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding MBNL1 protein (rAAVMyc-hMBNL1) and the left and right TAs were collected for total RNA preparation and assayed for recovery of the normal Clcn1 pre-mRNA splicing pattern. The results are shown in Figure 5A. The results show that the levels of the abnormal splicing products were decreased, **while the level of the normal splicing product was increased**, following rAAV1Myc-hMBNL1 injection.

Applicants direct the Examiner to Example 5, where to determine whether MBNL proteins can alter the splicing patterns of pre-mRNAs known to be abnormally regulated in DM1 striated muscle, GFP fusion proteins of all three MBNL proteins were transiently expressed with human and chicken cTNT minigenes in primary chicken skeletal muscle cultures. (p.30, lines 16 – 19). Applicants use Western blot analysis to investigate alternative splicing related to MBN, and show that **GFP-MBNL1, 2 and 3 strongly repressed inclusion of both human and chicken cTNT exon 5** in primary chicken skeletal muscle cultures, while expression of GFP to levels comparable to, or greater than, GFP-MBNL fusion proteins had no effect on splicing (shown in Figures 6A and 6B). page 32, line 24 – 26).

TABLE 2, beginning on page 45, shows a list of exons screened for abnormal regulation of alternative splicing in DM1 compared to control without neurologic disease, and includes APP, GRIN1 and MAPT.

Accordingly, the specification provides enablement for the claims as written.

**Claim Rejections 35 USC 103(a)**

The Examiner has indicated that claims 12 – 13 have been rejected under 35 USC 103(a) as being unpatentable over Snyder et al. (Human Gene Therapy (1997)) when taken with Miller et al. (EMBO J., 2000). Applicants respectfully disagree.

Claim 12 as presently written is directed to a pharmaceutical composition comprising a recombinant adeno-associated virus (rAAV) containing a transgene that

encodes a protein selected from the group consisting of: MBNL1, MBNL2 and MBNL3 and combinations thereof.

The Examiner argues that "Snyder teach utilizing a recombinant rAAV vector for gene transfer into adult immunocompetent mice and teach that AAV vectors efficiently and stably transducer post-mitotic muscle fibers and myoblasts in vivo." (Office Action, p.15). The Examiner admits that "Snyder do not specifically teach that the rAAV vector contains a transgene that encodes for MBNL1 (but) prior to the invention Miller teach the sequence of MBNL1 and...that MBNL1 is expressed in muscle tissue." (Office Action, p.16). Thus, the Examiner argues that "it would have been obvious...to modify the teachings of Snyder to produce an rAAVV vector containing a transgene that encoded MBNL1 with a reasonable expectation of success." (Office Action, p.16).

The Miller reference does not cure the defects of the Snyder reference. In particular, the Miller reference does not teach or suggest a pharmaceutical composition comprising a recombinant adeno-associated virus (rAAV) containing a transgene that encodes MBNL1. The Miller reference is directed to the identification of triplet repeat expansion (EXP) double-stranded (ds) RNA-binding proteins, which selectively associate with DM1 expansions. The Miller reference teaches purifying the EXP proteins and matches were found to the human 42 kDa MBNL protein. (p.4441). At best, the Miller reference teaches that " EXP proteins were structurally related to the Drosophila mbl proteins and expressed at a high level in skeletal muscle (which) suggested that EXP proteins might play key roles during the terminal differentiation of mammalian cell precursors." (p.4442). Nowhere does the Miller reference teach MBNL1, 2 or 3 or that any one of these would be useful in a pharmaceutical composition for administration.

Accordingly, it would not have been obvious to modify the teachings of Snyder to produce an rAAVV vector containing a transgene that encoded MBNL1 with a reasonable expectation of success.

Applicants respectfully request that the rejection be withdrawn.

## CONCLUSION

For the reasons provided, Applicant submits that all claims are allowable as written and respectfully requests early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

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Respectfully submitted,

By: Jonathan M. Sparks, Ph.D.
Registration No.: 53,624
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5543
Attorneys/Agents For Applicant